

**APPENDIX A**

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## IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re United States Patent Application of:	)	Docket No.:	020884-000008
Applicant(s): Juan Carlos Lacal Sanjuan, et al.	)	Examiner:	Zinna Northington Davis
Application No.: 10/597,095	)	Art Unit:	1625
U.S. Filing Date: September 29, 2006	)	Conf. No.:	5976
Title: DERIVATIVES OF PYRIDINE AND QUINOLINE	)	Customer No.:	24239

## DECLARATION UNDER 37 CFR §1.132 IN U.S. PATENT APPLICATION NO. 10/597,095

Mail Stop Amendment  
Commissioner for Patents  
P.O. Box 1450  
Alexandria, VA 22313-1450

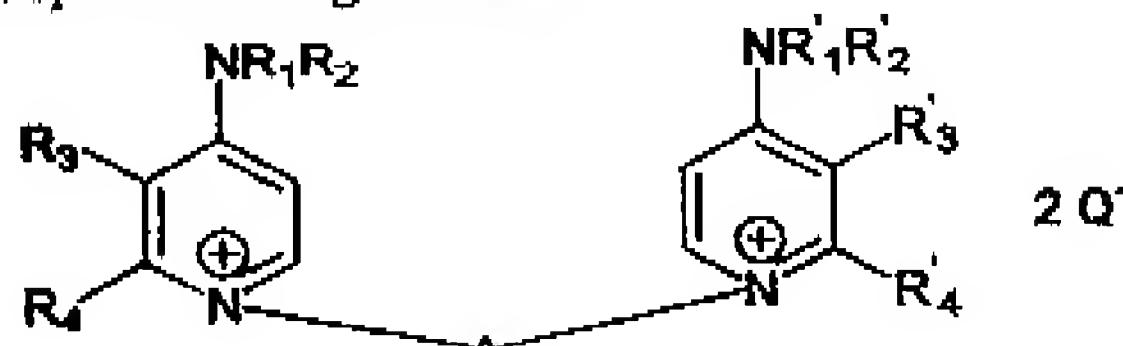
Sir:

JUAN CARLOS LACAL SANJUAN hereby declares:

1. THAT I am a co-inventor of the subject matter disclosed and elected in United States Patent Application No. 10/597,095 having a filing date of September 29, 2006 in the names of Juan Carlos Lacal Sanjuan, Joaquin Campos Rosa, Miguel Angel Gallo Meza and Antonio Espinosa Ubeda, and entitled, "DERIVATIVES OF PYRIDINE AND QUINOLINE," hereafter referred to as the "Application."
  
2. THAT the Application relates, in general, to compounds blocking phosphorylcholine biosynthesis by means of the selective blocking of the choline kinase enzyme in tumor cells or in cells affected by parasitic infection and therefore being applicable in the treatment of tumors and parasitic diseases or diseases produced by viruses, bacteria and fungi in animals including human beings. The claims being examined specifically relate to a compound as recited in claim 1:



## 1. A compound having formula I:



where

$Q^-$  represents the conjugate base of a pharmaceutically suitable organic or inorganic acid;

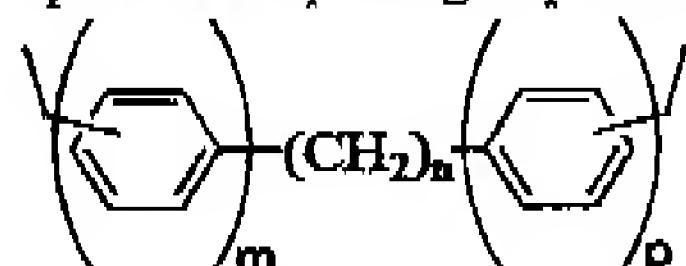
$R_1$  and  $R'_1$  represent, independently of each other, a radical selected from the group formed by H and  $C_{1-6}$  alkyl optionally substituted by trifluoromethyl, hydroxyl or alkoxy;

$R_2$  and  $R'_2$  represent, independently of each other, an aryl radical substituted by halogen, trifluoromethyl, hydroxyl,  $C_{1-6}$  alkyl, amino or alkoxy;

$R_3$  and  $R'_3$  represent, independently of each other, either a radical selected from the group formed by H, halogen, trifluoromethyl, hydroxyl, amino, alkoxy and  $C_{1-6}$  alkyl optionally substituted by trifluoromethyl, hydroxyl, amino or alkoxy, or together with  $R_4$  and  $R'_4$  respectively, and independently of each other, a  $-CH=CH-CH=CH-$  radical optionally substituted by halogen, trifluoromethyl, hydroxyl,  $C_{1-6}$  alkyl, amino or alkoxy;

$R_4$  and  $R'_4$  represent, independently of each other, either a radical selected from the group formed by H and  $C_{1-6}$  alkyl optionally substituted by halogen, trifluoromethyl, hydroxyl, amino or alkoxy, or together with  $R_3$  and  $R'_3$  respectively, and independently of each other, a  $-CH=CH-CH=CH-$  radical optionally substituted by halogen, trifluoromethyl, hydroxyl,  $C_{1-6}$  alkyl, amino or alkoxy; and

A represents a spacer group having the following formula:



wherein m, n and p represent integers which can have the following values: m = 1; n = 0, 1-10; p = 0, 1; with the condition that m, n and p do not take the value of zero at the same time.

3. THAT Exhibit A attached herewith is a report including comparative activity results supervised by the undersigned. The comparative experiments are being included to show that claim 1, as presently pending, including the limitation that " $R_2$  and  $R'_2$  represent, independently of each other, an aryl radical substituted by halogen, trifluoromethyl, hydroxyl,  $C_{1-6}$  alkyl, amino or

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*alkoxy*" present a dramatically improved *in vivo* activity and toxicity, compared to the corresponding derivatives wherein the radical at R<sub>2</sub> and R<sub>2'</sub> is non-substituted.

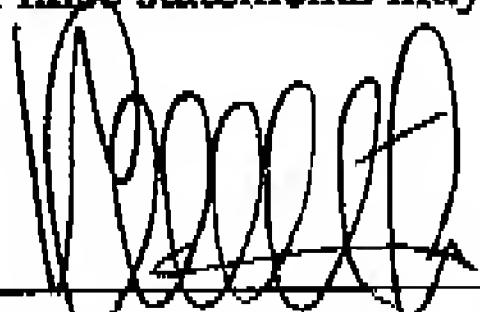
Campos et al.<sup>1</sup> (compound 3k) (hereinafter C1), Conejo-Garcia et al.<sup>2</sup> (compounds 5 and 9) (hereinafter C2) and Campos et al.<sup>3</sup> (compounds 57-59) (hereinafter C3) can be considered as the closest prior art documents.

The above-mentioned compounds described in C1-C3 contain a similar core structure as the compounds of the invention, except for the radical at position R<sub>2</sub> and R<sub>2'</sub>. As previously discussed, R<sub>2</sub> and R<sub>2'</sub> in the compounds in C1-C3 are unsubstituted aryl radicals, whereas in the present application R<sub>2</sub> and R<sub>2'</sub> are substituted aryl groups.

Turning to the Comparative Results provided herein, it can be clearly seen that non-substituted compound 2 shows a remarkably higher toxicity (lower Lethal Dose value) than the substituted compounds of the present invention (compounds 1, 3-10). In addition, non-substituted compound 2 presents very low (5D schedule) or no (1D schedule) *in vivo* antitumoral activity, while the presently claimed compounds (compounds 1, 3-10) show notably high *in vivo* antitumoral activity according to both 5D and 1D schedule.

4. THAT I offer Exhibit A with this Declaration as evidence of unexpected results achieved as a result of substituting the aryl radicals.

As a below-named declarant, I hereby declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements are made with the knowledge that willful false statements, and the like, so made are punishable by fine or imprisonment, or both, under Section 1001 or Title 18 of the United States Code and that such willful false statements may jeopardize the validity of the application or any patent issued thereon.



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JUAN CARLOS LACAL SANJUAN

Date December 14, 2009<sup>1</sup> Farmaco, 58(3) (2003) 221-229.<sup>2</sup> Eur. J. Med. Chem., 38(1) (2003) 109-116.<sup>3</sup> Bioorg. Med. Chem., 10(7) (2002) 2215-2231.

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**EXHIBIT A**  
**COMPARATIVE ACTIVITY RESULTS**

**in Vivo assays of Antitumoral Activity**

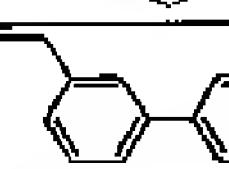
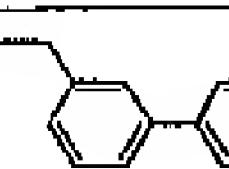
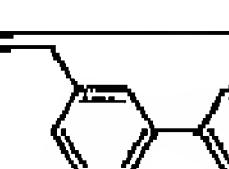
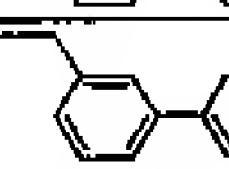
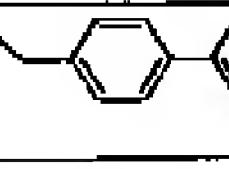
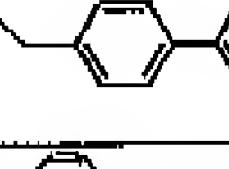
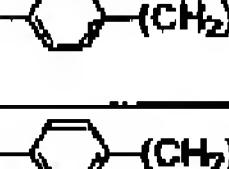
The *in vivo* antitumoral activity of the compounds was tested using a human xenograft model. HT-29 cells derived from a human colon adenocarcinoma were subcutaneously injected in both flanks of nude mice (Swiss, nu/nu). When tumours reached approximately a volume of 0.1 cm<sup>3</sup>, mice are treated with the indicated amounts of each compound, and those used as controls are injected only with the solvent used for each drug. The increase in volume of the tumours is monitored twice per week. Tumour volume is calculated using the following formula: V = (D<sup>2</sup>d<sub>2</sub>)/2, where D is the biggest diameter and d is the smallest one. In order to identify the most powerful compounds, two different schedules of treatment were followed:

## 1.- Standard treatment schedule:

**5 DAYS TREATMENT - 9 DAYS REST - 5 DAYS TREATMENT**  
**(DURATION OF THE PRECLINICAL TRIAL: 5 WEEKS)**

## 2.- Restrictive treatment schedule:

**1 DAY PER WEEK**  
**(DURATION OF THE PRECLINICAL TRIAL: 4 WEEKS)**

Comp. No.	R <sub>1</sub> , R <sub>2</sub>	NR <sub>1</sub> R <sub>2</sub>	A	LD <sub>50</sub> (mg/kg)	In vivo activity SD schedule % Day 22	In vivo activity 1D schedule % Day 21
1	H, H	-N(Me)C <sub>6</sub> H <sub>4</sub> Cl		23.2	52.5 %	42.5 %
3	H, H	-N(Me)C <sub>6</sub> H <sub>4</sub> Cl		>25	58.5 %	31 %
4	H, H	-N(Me)C <sub>6</sub> H <sub>3</sub> Cl <sub>2</sub>		22.4	55 %	38 %
5	-(CH=CH) <sub>2</sub>	-N(Me)C <sub>6</sub> H <sub>4</sub> Cl		23	65.2 %	36 %
6	-C <sup>6</sup> H=C <sup>6</sup> H-C <sup>6</sup> Cl=C <sup>6</sup> H-	-N(Me)C <sub>6</sub> H <sub>4</sub> Cl		39.9	68 %	62 %
7	-(CH=CH) <sub>2</sub>	-N(Me)C <sub>6</sub> H <sub>4</sub> Cl		10.9	63.3 %	75 %
8	-C <sup>6</sup> H=C <sup>6</sup> H-C <sup>6</sup> Cl=C <sup>6</sup> H-	-N(Me)C <sub>6</sub> H <sub>4</sub> Cl		12.5	46.4 %	35.3 %
9	-(CH=CH) <sub>2</sub>	-N(Me)C <sub>6</sub> H <sub>4</sub> Cl		24.5	55.2 %	31.5 %
10	-C <sup>6</sup> H=C <sup>6</sup> H-C <sup>6</sup> Cl=C <sup>6</sup> H-	-N(Me)C <sub>6</sub> H <sub>4</sub> Cl		20	58 %	59.2 %
2 (comparative)	H, H	-		4.5	5 %	0

